# Assessing biological soil quality with chloroform fumigation-incubation: Why subtract a control?

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[1]*US Department of Agriculture-Agricultural Research Service, J. Phil Campbell Sr. Natural Resources Conservation Center, 1420 Experiment Station Road, Watkinsville, GA 30677 (E-mail: afranz@arches.uga.edu); and* [2]*Texas Agricultural Experiment Station, Department of Soil & Crop Sciences, Texas A&M University, College Station, TX 77843.*

Franzluebbers, A. J., Haney, R. L., Hons, F. M. and Zuberer, D. A. 1999. **Assessing biological soil quality with chloroform fumigation-incubation: Why subtract a control?** Can. J. Soil Sci. **79**: 521–528. Microbial biomass, as part of the active pool of soil organic matter, is critical in decomposition of organic materials, nutrient cycling, and formation of soil structure. We evaluated **chloroform fumigation-incubation with subtraction of a control (CFI/F–C)** and **without subtraction of a control (CFI/F)** as methods to assess biological soil quality. Relationships between CFI/F and potential C mineralization, particulate organic C, and soil organic C were stronger ($r^2 = 0.86 \pm 0.07$, $n = 232$) than those between CFI/F–C and the same soil C pools ($r^2 = 0.25 \pm 0.09$) in soils from Georgia. From published data, relationships of CFI/F with potential C mineralization and soil organic C were stronger than those of chloroform fumigation-extraction and substrate-induced respiration with these soil C pools. Effects of land management on biological soil quality using CFI/F were consistent with those determined using other soil C pools as response variables. However, land management effects on biological soil quality using CFI/F–C were either contrary to those using other soil C pools or not detectable because of greater inherent variability in CFI/F–C. Chloroform fumigation-incubation without subtraction of a control is a robust and reliable method to assess biological soil quality under a wide range of soil conditions.

**Key words**: Active soil carbon, chloroform fumigation-extraction, microbial biomass, soil organic matter, soil quality, substrate-induced respiration

Franzluebbers, A. J., Haney, R. L., Hons, F. M. et Zuberer, D. A. 1999. **Évaluation de la qualité biologique du sol par la méthode de fumigation au chloroforme-incubation: pourquoi soustraire les valeurs témoins?** Can. J. Soil Sci. **79**: 521–528. Participant intégralement au pool actif de la matière organique du sol, la biomasse microbienne joue un rôle critique dans la décomposition des matières organiques ainsi que dans les transformations des éléments nutritifs et dans la formation de la structure du sol. Nous avons comparé l'efficacité de la méthode de fumigation au chloroforme suivie d'incubation, avec (FCI/F-C) et sans substraction (FCI/F) des valeurs témoins, dans l'évaluation de la qualité biologique du sol. Les rapports obtenus entre FCI/F et le C potentiellement minéralisable, le C organique particulaire et le C organique du sol, étaient plus étroit ($r^2 = 0{,}25 \pm 0{,}07$, $n = 232$) que ceux obtenus entre FCI/F-C et les mêmes pools de C du sol ($r^2 = 0{,}25 \pm 0{,}09$), dans les sols de Georgie (USA). À partir des données publiées, il ressort que les rapports entre FCI/F, d'une part, et le C potentiellement minéralisable et le C organique du sol, d'autre part, étaient plus serrés que ceux obtenus pour ces mêmes pools avec les méthodes de la fumigation au chloroforme suivie d'extraction, et de la respiration induite par le substrat. Les effets de la conduite des terres sur la qualité biologique du sol, mesurés par FCI/F, concordaient avec ceux déterminés avec d'autres pools de C comme variables. Par comparaison, ces mêmes effets mesurés par FCI/F-C étaient, soit contraire à ceux utilisant ces autres pools de C, soit simplement indétectables en raison de la plus large variabilité inhérente à cette méthode. La fumigation au chloroforme suivie d'incubation, sans substraction des valeurs témoins apparaît donc comme une méthode solide et sûre d'évaluation de la qualité biologique du sol dans un large éventail de conditions de sols.

**Mots clés**: Carbone actif du sol, fumigation au chloroforme-extraction, biomasse microbienne, matière organique du sol, qualité du sol, respiration induite par le substrat

Soil microbial biomass is an important component of soil quality assessment because of its important roles in nutrient dynamics, decomposition of natural and synthetic organic amendments, and physical stabilization of aggregates. Numerous methods are now available to estimate this active pool of soil organic matter, including variants of **chloroform fumigation-incubation (CFI)** (Jenkinson and Powlson 1976; Chaussod and Nicolardot 1982; Voroney and Paul 1984; Smith et al. 1995), **chloroform fumigation-extraction (CFE)** (Brookes et al. 1985; Vance et al. 1987a), **substrate-induced respiration (SIR)** (Anderson and Domsch 1978; Smith et al. 1985), and adenosine triphosphate (Webster et al. 1984). Several reviews describe the advantages and disadvantages of some of these methods (Jenkinson and Ladd 1981; Smith and Paul 1990; Alef 1993; Horwath and Paul 1994; Martens 1995).

Chloroform fumigation-incubation has been the most common method for determining microbial biomass C (Martens 1995). It is the standard by which most other newer methods have been compared. However, negative microbial biomass estimates have been calculated when the control evolves more $CO_2$ than the fumigated sample, which can occur when soils are extremely acidic or have received recent organic amendments (Martens 1985; Vance et al. 1987b). Due to this potential aberration, debate has continued as to whether a control should be subtracted (Voroney and Paul 1984) or which control should be subtracted (Jenkinson and Powlson 1976; Chaussod and Nicolardot

1982; Smith et al. 1995) when using CFI. Despite the unreliability of CFI/F–C under certain situations, alternative methods for estimating microbial biomass (i.e., CFE, SIR, and adenosine triphosphate) have been calibrated against it. The question of subtracting a control using CFI is, therefore, of utmost importance in order for these alternative methods to accurately describe microbial biomass.

In general, soil microbial biomass C is a relatively small component (1–10%) of the total organic C pool (Fig. 1). As the total organic C pool expands or contracts due to changes in C inputs to the soil, the microbial pool also expands or contracts, although not necessarily in exact unison on a short time scale (Powlson et al. 1987; Franzluebbers et al. 1994a). However, a close relationship between microbial biomass and potential C mineralization should exist within soils from the same climate because a natural balance exists among substrate availability and utilization and microbial population dynamics in relatively stable ecosystems. Since an absolute measure of soil microbial biomass C is not available, comparison of microbial biomass estimates with other soil C and N pools should provide useful information to test the adequacy of various methods. Relationships between CFI/F and other soil C and N pools (i.e., mineralizable, particulate, and total) were much stronger in several data sets from Texas, Georgia, and Alberta/British Columbia than between CFI/F–C and these soil C and N pools (Franzluebbers et al. 1999). These results strongly suggest that CFI/F should be preferred over the original CFI/F–C as an indicator of biologically active soil C or microbial biomass. However, the reliability of CFI/F and CFI/F–C to assess biological soil quality needs to be further investigated under variations in land management conditions known to alter soil organic matter dynamics.

Our objective was to further test whether subtraction of a control in CFI is appropriate to estimate microbial biomass by (i) comparing CFI/F and CFI/F–C methods to a suite of other soil C and N pools from recently sampled long-term experiments, (ii) comparing CFI/F, CFI/F–C, CFE, and SIR methods to steady-state C mineralization and soil organic C from published studies, and (iii) comparing the ability of CFI/F and CFI/F–C methods to detect management-induced changes in active soil C pools, which help define biological soil quality.

## MATERIALS AND METHODS

### Objective 1

Five soil samples were collected from a Coastal bermudagrass (*Cynodon dactylon* L.) pasture on Typic Kanhapludults in Georgia at depths of 0–20, 20–40, and 40–60 mm in May 1996 to yield a wide range in potentially mineralizable C. Soils of the same classification were collected from under tall fescue (*Festuca arundinacea* Schreb.) pastures at depths of 0–25, 25–75, 75–150, and 150–300 mm in January and February 1997.

We modified the original CFI method proposed by Jenkinson and Powlson (1976b) to estimate soil microbial biomass C. Smaller quantities (15 to 60 g) of oven-dried soil (55°C) were placed into graduated bottles, moistened to 50% water-filled pore space, placed into 1-L canning jars in the presence of 10 mL of 1.0 M NaOH, and incubated at 25 ± 1°C for up to 24 d. A pre-incubation period of 10 d allowed the soil to establish a steady-state level of microbial activity and has been shown to yield estimates of microbial biomass similar to those from field-moist soil (Franzluebbers et al. 1996a; Franzluebbers 1999). $CO_2$-C evolved during 10 d following fumigation was determined by titration of alkali with 1.0 M HCl. Flush of $CO_2$-C evolved following fumigation was calculated with subtraction of a 10-d control (calculated from 10 to 24 d rate) as proposed in the original method by Jenkinson and Powlson (1976) and without subtraction of a control as suggested by Voroney and Paul (1984).

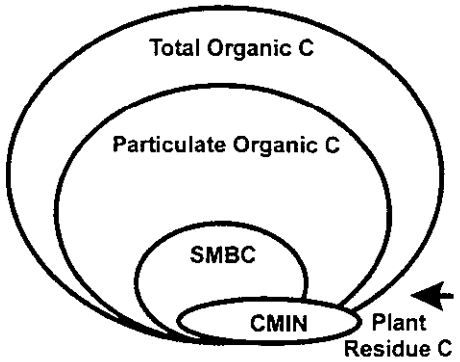


**Fig. 1.** Conceptual diagram of the relative size and relationships of soil C pools. Soil microbial biomass C (SMBC) is positioned as an intermediate pool that feeds (expressed as C mineralization, CMIN) upon actively cycling plant residue C and slowly cycling particulate and total organic C pools.

Steady-state C mineralization from the unfumigated soil was determined during the 10- to 24-d period using methodology described above. Soil organic C and N were determined by dry combustion without estimation of carbonates since all soils had pH less than 6.5. Particulate organic C and N were determined by shaking the oven-dried (55°C, 72 h) fumigated sample previously used for microbial biomass determination with 0.01 M $Na_4P_2O_7$ for 16 h, collecting the sand plus organic matter retained on a 0.06-mm screen, oven-drying (55°C, 72 h), weighing, grinding to a fine powder, and determining the C and N concentrations using dry combustion. Net N mineralization was determined from the difference in inorganic N ($NO_3$-N + $NO_2$-N + $NH_4$-N) concentration between 0 and 24 d of incubation using Cd reduction and salicylate autoanalyzer techniques in 2 M KCl extracts (Bundy and Meisinger 1994). Regression analyses were performed using SAS (SAS Institute, Inc. 1990). Relationships of CFI/F and CFI/F–C with various pools of soil organic matter were evaluated by comparing coefficients of determination ($r^2$).

### Objective 2

Published data were selected to assess the relationships of CFI/F and CFI/F–C with soil organic C, steady-state C mineralization, CFE, and SIR. Steady-state C mineralization

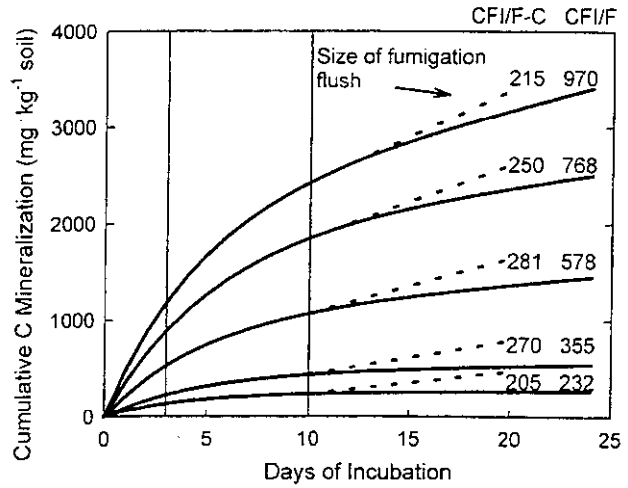


**Fig. 2.** Cumulative C mineralization during 24 d of incubation among five soils under bermudagrass in Georgia. Fumigation flush is from a duplicate sample fumigated at 10 d and $CO_2$-C evolved during the subsequent 10 d. CFI/F–C is chloroform fumigation-incubation with subtraction of a 10-d control and CFI/F is without subtraction of a control.

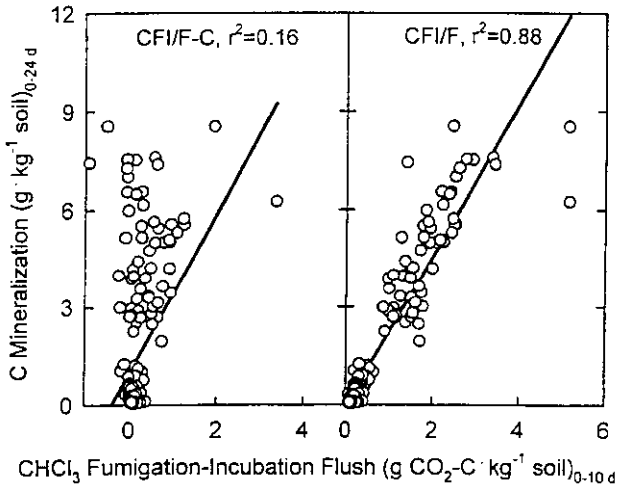


**Fig. 3.** Relationship of C mineralization with chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) in Typic Kanhapludults in Georgia. Soils were collected from depths of 0–25, 25–75, 75–150, and 150–300 mm under tall fescue pasture with low and high endophyte and low and high fertilization (data from Franzluebbers et al. 1999b).

was taken as the $CO_2$-C evolved during the 10- to 20-d period if available or otherwise during the 0- to 10-d period. In the study of Sparling and Zhu (1993), where some data were available from the 10- to 20-d period, we selected only the 0- to 10-d period to be consistent within the study.

### Objective 3

Unpublished and published data were selected to illustrate the response in CFI with and without subtraction of a control to changes in land management systems. Results of each method were compared with those of other active, passive, and total pools of organic matter.

## RESULTS AND DISCUSSION

### Relationship of CFI/F and CFI/F–C with Other Soil Organic Pools

By design, potential C mineralization of the soil samples collected under bermudagrass pasture exhibited widely different (up to 30-fold) rates (Fig. 2). With an initial flush of $CO_2$ following rewetting of dried soil, rates of mineralization stabilized for all soils after 3 to 10 d of incubation. Relationship of CFI/F was strong ($r^2$ = 0.99) with both cumulative C mineralization during 24 d and steady-state rate of C mineralization. However, no relationship was observed between CFI/F–C and these two C mineralization estimates ($r^2$ = 0.03). It seems unreasonable that soils ranging in steady-state C mineralization from 2 to 71 mg $kg^{-1}$ $d^{-1}$ would have essentially the same microbial biomass (as was estimated with CFI/F–C). Estimation of microbial biomass with CFI/F was reasonable in relative comparison among soils with very different C mineralization capacities.

The relationship of CFI/F with cumulative C mineralization during 24 d ($r^2$ = 0.88, $n$ = 232) was strong in a set of soil samples collected from under tall fescue pasture in Georgia (data from Franzluebbers et al. 1999b; Fig. 3). However, there was only a weak relationship of CFI/F-C with cumulative C mineralization. The relationship of CFI/F was also stronger with other soil C and N pools than that of CFI/F–C with these pools, including soil organic C ($r^2$ = 0.78 with CFI/F, $r^2$ = 0.25 with CFI/F-C), soil organic N ($r^2$ = 0.77 with CFI/F, $r^2$ = 0.27 with CFI/F–C), particulate organic C ($r^2$ = 0.92 with CFI/F, $r^2$ = 0.33 with CFI/F–C), particulate organic N ($r^2$ = 0.86 with CFI/F, $r^2$ = 0.31 with CFI/F-C), and net N mineralization during 24 d ($r^2$ = 0.74 with CFI/F, $r^2$ = 0.51 with CFI/F–C). Therefore, interpretation of pasture management and environmental characteristics on the standing stock of microbial biomass using CFI/F was consistent with other soil C and N pools, but not using CFI/F–C. This same discrepancy between CFI/F and CFI/F–C in relationships with soil C and N pools was reported for several other data sets from Texas, Georgia, and Alberta/British Columbia (Franzluebbers et al. 1999a).

### Relationships of CFI/F and CFI/F–C with Other Microbial Biomass Methods

From eight studies with data available to compare CFI and CFE ($n$ = 108), the relationship of CFI/F with CFE was weaker than that of CFI/F–C with CFE (Fig. 4). The strength of the relationship between CFI/F–C and CFE is perhaps expected, since CFE was originally calibrated against CFI/F–C for most of the 10 soils (CFI/F was used for two of the soils) reported in Vance et al. (1987a). Despite the weaker overall relationship between CFI/F and CFE, relationships among soil samples within individual studies averaged 0.82 ± 0.17 between CFE and CFI/F and 0.80 ± 0.20 between CFE and CFI/F–C. The relationship of CFI/F with steady-state C mineralization in these studies was much stronger than that of CFE with steady-state C mineralization

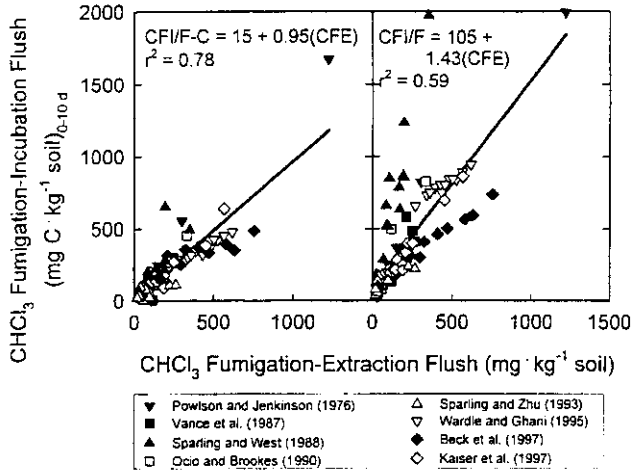


**Fig. 4.** Relationship of chloroform fumigation-extraction (CFE) with chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) from eight published studies.

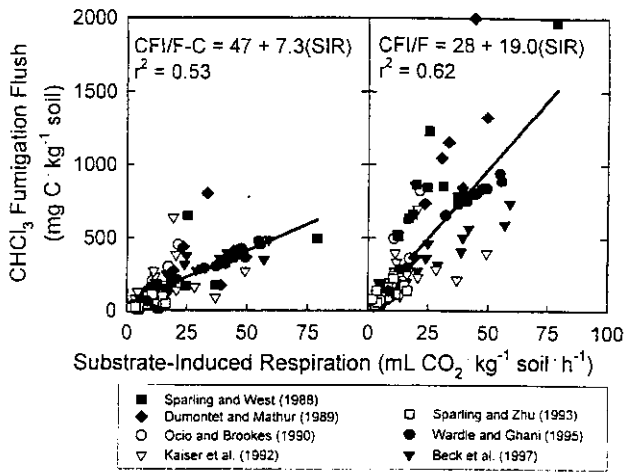


**Fig. 6.** Relationship of substrate-induced respiration (SIR) with chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) from seven published studies.

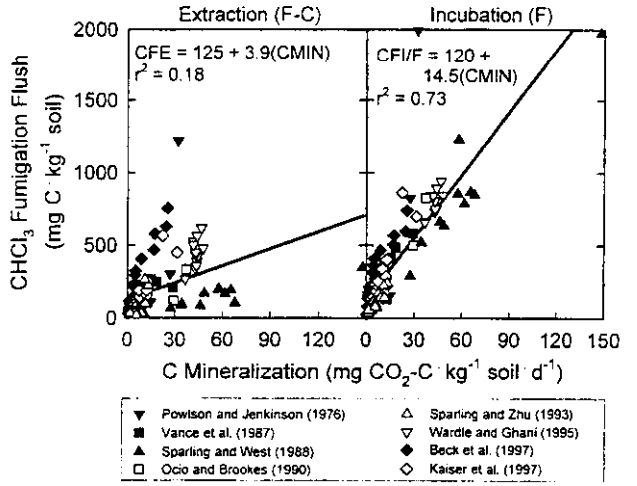


**Fig. 5.** Relationship of steady-state C mineralization with chloroform fumigation-extraction (CFE) and chloroform fumigation-incubation without subtraction of a control (CFI/F) from eight published studies.

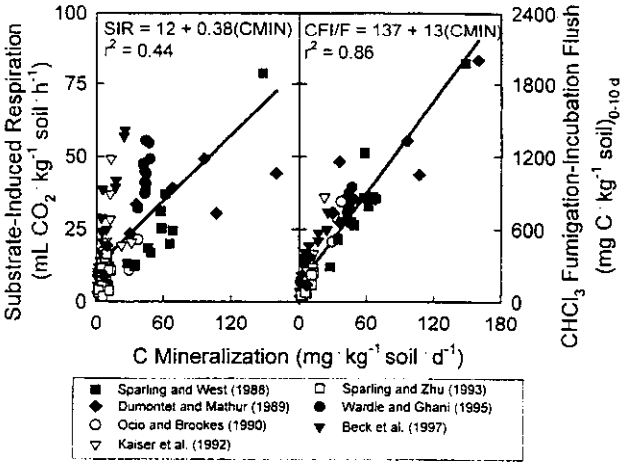


**Fig. 7.** Relationship of steady-state C mineralization with substrate-induced respiration (SIR) and chloroform fumigation-incubation without subtraction of a control (CFI/F) from seven published studies.

(Fig. 5). In the same manner, the relationship of CFI/F with soil organic C was stronger ($r^2 = 0.68$) than that of CFE with soil organic C ($r^2 = 0.25$). Relationships of CFI/F–C with steady-state C mineralization ($r^2 = 0.21$) and soil organic C ($r^2 = 0.20$) were both weak. Therefore, CFI/F was more consistent with other soil C pools (i.e., steady-state C mineralization and soil organic C) than was either CFE or CFI/F-C.

From seven studies with data available to compare CFI and SIR ($n = 106$), the relationship of CFI/F was somewhat stronger with SIR than CFI/F-C with SIR (Fig. 6). Relationships among soils within individual studies were also somewhat stronger between CFI/F and SIR ($r^2 = 0.68 \pm 0.29$) than between CFI/F–C and SIR ($r^2 = 0.54 \pm 0.36$). The relationship of CFI/F with steady-state C mineralization in these studies was stronger than that of SIR with steady-state C mineralization (Fig. 7). Similarly, the relationship of CFI/F with soil organic C was stronger ($r^2 = 0.60$) than that of SIR with soil organic C ($r^2 = 0.41$). Close correlation between CFI and SIR may not be expected, since SIR measures the metabolically active subset of total microbial biomass measured by CFI.

We have shown that CFI/F was more consistently related with other soil C pools than was CFI/F–C, CFE, or SIR. It has also been previously shown that in comparison with the original CFI/F–C method, CFI/F forms stronger relationships with other biochemical and biophysical soil properties, including adenosine triphosphate, direct counting, net N mineralization, CFI flush of N, particulate organic N,

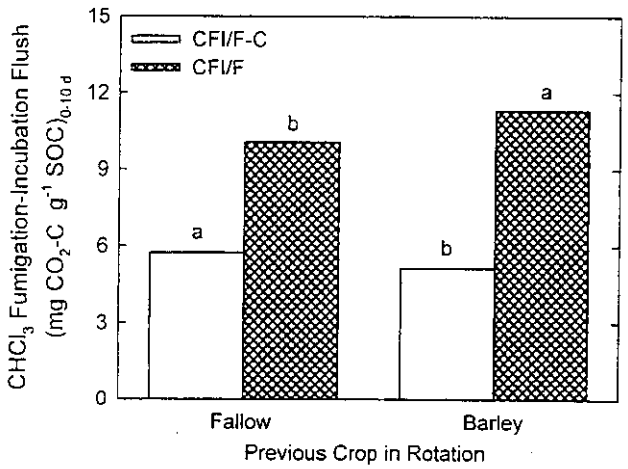


**Fig. 8.** Effect of previous crop on chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) from a Typic Natriboralf in Alberta sampled to a depth of 0–50 mm (data from Franzluebbers and Arshad 1996a).

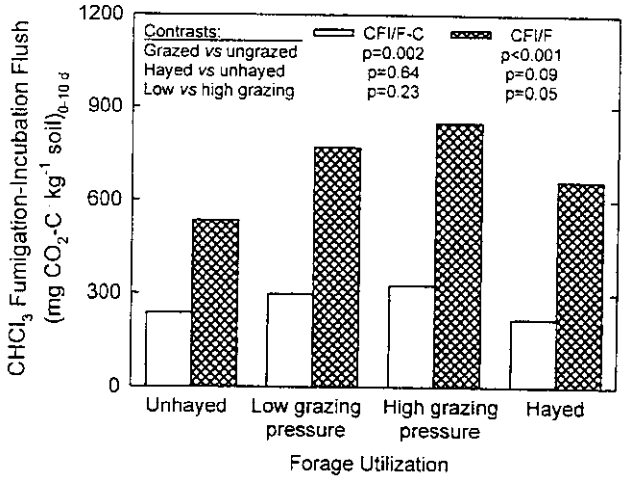


**Fig. 10.** Effect of forage utilization regime on chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) from a Typic Kanhapludult in Georgia sampled to a depth of 0–20 mm.

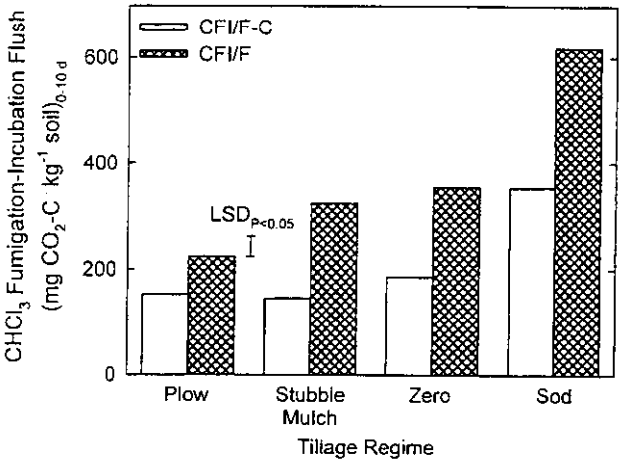


**Fig. 9.** Effect of tillage regime on chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) from a Pachic Haplustoll in Nebraska sampled to a depth of 0–100 mm (data from Follett and Schimel 1989).

mean-weight diameter of water-stable aggregates, and total porosity (Franzluebbers et al. 1999a). From these relationships among various biochemical and biophysical soil properties, it becomes evident that CFI/F is a more robust procedure to estimate the biologically active component of soil organic matter than CFI/F–C.

## Comparison of CFI/F and CFI/F–C in Biological Soil Quality Assessment

On a Typic Natriboralf (Solodized Solonetz) in Alberta, the flush of $CO_2$ using CFI/F–C in the surface 50 mm of a canola (*Brassica campestris* L.)–wheat (*Triticum aestivum* L.)–barley (*Hordeum vulgare* L.)–fallow rotation was 11% less ($P = 0.04$) following barley than following fallow (data from Franzluebbers and Arshad 1996a; Fig. 8). However using CFI/F, the flush of $CO_2$ was 10% greater ($P < 0.01$) following barley than following fallow. Steady-state C mineralization was 43% greater ($P < 0.01$) following barley than following fallow. We hypothesize that crop residue input following barley should have provided readily mineralizable C substrates for growth rather than decline of soil microbial biomass C.

On a Pachic Haplustoll in Nebraska, the flush of $CO_2$ using CFI/F in the surface 100 mm of a wheat–fallow rotation increased with decreasing level of intensity of disturbance with tillage (data from Follett and Schimel 1989; Fig. 9). Incorporation of crop residues generally favors in situ decomposition and long-term loss of substrates available for maintenance of microbial biomass (Doran and Linn 1994). Cumulative C mineralization during 20 d of incubation was very closely related to CFI/F ($r^2 = 0.97$). With CFI/F as response variable, microbial biomass under stubble–mulch tillage was 45% greater, under zero tillage was 58% greater, and under sod was 2.8-fold greater than under plow tillage. Tillage effects were similar to CFI/F with cumulative C mineralization as response variable (i.e., under stubble–mulch tillage was 87% greater, under zero tillage was 2.1-fold greater, and under sod was 3.3-fold greater than under plow tillage). However, tillage effects were much subdued using CFI/F–C as response variable (i.e., under stubble–mulch tillage was 5% lower, under zero tillage was 22% greater, and under sod was 1.3-fold greater than under plow tillage). Different interpretations of management-induced changes in active soil C pools resulted with choice of method.

On a Typic Kanhapludult in Georgia, CFI/F and CFI/F–C in the surface 20 mm both indicated that cattle grazing on coastal bermudagrass increased microbial biomass ~35% compared with hayed or unhayed bermuda (unpublished data 1998; Fig. 10). Coefficients of variation were 24% for

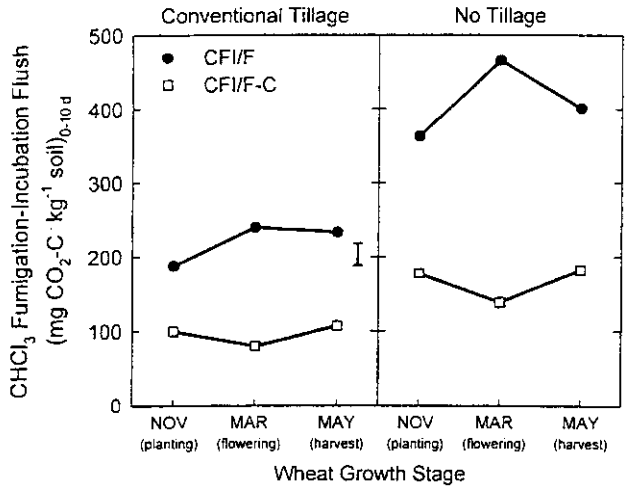


**Fig. 11.** Effect of wheat growth stage on chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) from a Fluventic Ustochrept in Texas sampled to a depth of 0–50 mm (data from Franzluebbers et al. 1994b).

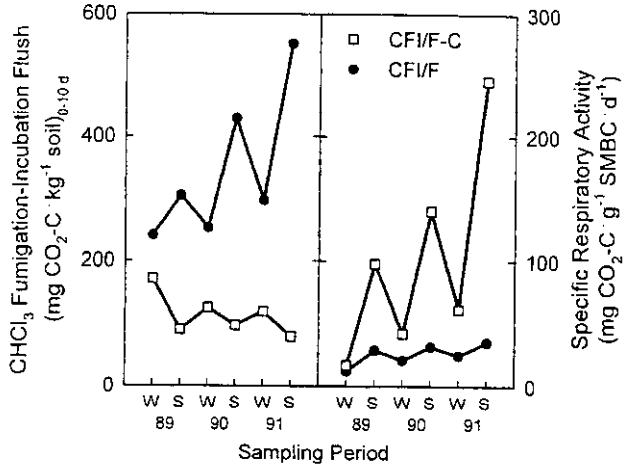


**Fig. 12.** Effect of sampling time [winter (W) following soybean harvest and summer (S) following wheat harvest] in a wheat/soybean double cropping system on chloroform fumigation-incubation with (CFI/F–C) and without subtraction of a control (CFI/F) and specific respiratory activity from a Typic Argiudoll in Argentina sampled to a depth of 0–100 mm (data from Alvarez et al. 1995).

CFI/F and 48% for CFI/F–C. Hayed bermudagrass contained 23% greater ($P < 0.1$) microbial biomass using CFI/F than unhayed bermudagrass. Also, high grazing pressure contained 10% greater ($P < 0.1$) microbial biomass using CFI/F than low grazing pressure. No statistical differences in these contrasts occurred using CFI/F–C. Results from CFI/F were consistent with those of cumulative C mineralization during 24 d of incubation, where treatment differences in the three contrasts were of the same magnitude and significance.

On a Fluventic Ustochrept in Texas, the flush of $CO_2$ using CFI/F in the surface 50 mm of continuous wheat increased from planting to flowering and was higher at harvest than at planting (data from Franzluebbers et al. 1994b; Fig. 11). It would be reasonable to hypothesize that soil microbial biomass C was greater at flowering and immediately at the end of the wheat growing season when root and residue inputs were high compared with the beginning of the wheat growing season when 5 mo of fallow passed without C inputs. Seasonal dynamics of CFI/F–C, however, indicated that microbial biomass declined during the growing season with recovery to original levels at the end of the growing season. In many cropping systems, which are rich in nutrients and poor in food-web diversity, plant inputs generally drive soil microbial pool sizes and activities. It should be noted that increases in plant production in natural systems do not always lead to increased microbial biomass, because of nutrient competition between plants and microorganisms (Okano et al. 1991) and because of plant resource competition between soil fauna and microorganisms (Wardle et al. 1998).

On a Typic Argiudoll in the Argentine Rolling Pampa, CFI/F in the surface 100 mm of a wheat–soybean [*Glycine max* L. (Merr.)] double-cropping system was greater following wheat (sampled in summer) than following soybean (sampled in winter) and increased at a rate of ~98 mg $kg^{-1}$ $yr^{-1}$ during the initial 3 yr (data from Alvarez et al. 1995; Fig. 12). Specific respiratory activity of microbial biomass (using CFI/F) was also greater in summer than in winter and increased ~7 mg $g^{-1}$ $d^{-1}$ each year from an initial level of ~17 mg $g^{-1}$ $d^{-1}$. The initial level and seasonal variation in specific respiratory activity using CFI/F in this cited study are comparable to those (17 ± 3 mg $g^{-1}$ $d^{-1}$) found in a similar long-term cropping system in Texas on a Fluventic Ustochrept with similar variations in summer and winter soil temperatures (Franzluebbers et al. 1996b). Interpretation of data changed dramatically if CFI/F–C was used to estimate soil microbial biomass C. Rather than CFI flush increasing with time as with CFI/F, CFI/F–C decreased at a rate of ~23 mg $kg^{-1}$ $yr^{-1}$ upon adoption of the high-residue-input, double cropping system. Further, specific respiratory activity using CFI/F–C increased at a rate nearly 10 times greater than that using CFI/F. On the last sampling date, specific respiratory activity using CFI/F–C suggested that the entire microbial biomass was turning over once every 4 d (~250 mg $g^{-1}$ $d^{-1}$). Such rapid turnover in soil is unlikely given the heterogeneous distribution of substrates available for microbial growth and the large dormant population protected from decomposition. Less rapid microbial turnover in soil has been reported for a range of soils in North America (40 to 60 mg $g^{-1}$ $d^{-1}$; Insam 1990), for a Luvisol and a Phaeozem in Germany (~40 mg $g^{-1}$ $d^{-1}$; Anderson and Domsch 1986); for a Fluventic Ustochrept in Texas (6 to 24 mg $g^{-1}$ $d^{-1}$; Franzluebbers et al. 1996b), and for Mollic and Typic Cryoboralfs in Alberta/British Columbia (9 to 38 mg $g^{-1}$ $d^{-1}$; Franzluebbers and Arshad 1996b).

Large temporal variations in soil microbial biomass have been linked to differences in soil moisture, temperature, and C inputs (Wardle 1998). However, relatively small temporal

**Table 1. Temporal variation in soil microbial biomass C using chloroform fumigation with (CFI/F–C) and without (CFI/F) subtraction of a control in published studies**

| Study | *n* | Ecosystem | Temporal variation (%) CFI/F–C | CFI/F |
|---|---|---|---|---|
| Srivastava and Singh (1989) | 6 | Cropland | 24 | 18 |
| Srivastava and Singh (1989) | 6 | Forest | 67 | 18 |
| Patra et al. (1990) | 12 | Cropland | 8 | 5 |
| Patra et al. (1990) | 11 | Pasture | 9 | 9 |
| Luizao et al. (1992) | 13 | Pasture | 34 | 24 |
| Franzluebbers et al. (1994b) | 54 | Cropland | 13 | 10 |
| Franzluebbers et al. (1995a) | 24 | Cropland | 10 | 7 |
| Franzluebbers et al. (1995b) | 72 | Cropland | 15 | 10 |
| Patra et al. (1995) | 24 | Cropland | 22 | 17 |
| Franzluebbers et al. (1996b) | 72 | Cropland | 14 | 8 |

variations in soil microbial biomass have been reported, and were expected based on theoretical considerations, under a stable grassland ecosystem (Patra et al. 1990). Whether or not a control is subtracted using CFI can help explain why some studies report large and others small temporal variations in soil microbial biomass. Temporal variation in soil microbial biomass C (measured as the coefficient of variation among samples from the same ecosystem collected at different times of the year) was almost always greater using CFI/F–C than using CFI/F in several published studies (Table 1). Temporal variation in these studies was 65 ± 76% greater using CFI/F–C than using CFI/F. Using CFI/F, soil microbial biomass C was more temporally stable than steady-state C mineralization, which fluctuated greatly due to pulses of readily decomposable C inputs that may not have contributed immediately to increases in microbial biomass (Franzluebbers et al. 1996b). This difference in temporal stability between C mineralization and microbial biomass is in agreement with the theoretical considerations offered by Patra et al. (1990). To illustrate the effect of soil disturbance on stability of active soil C pools, temporal variation in steady-state C mineralization was threefold greater than that in soil microbial biomass C under conventional tillage, but temporal variation in steady-state C mineralization was similar to that in soil microbial biomass C under no tillage (Franzluebbers et al. 1996b).

## CONCLUSIONS

This review of methodology strongly supports CFI/F as a more robust technique to estimate soil microbial biomass than CFI/F–C, as well as many of the current alternative methods, including CFE and SIR. Chloroform fumigation-incubation without subtraction of a control should also be considered a more sensitive and reasonable indicator of management-induced changes in soil organic matter quantity and quality than the original CFI/F–C. It is rare to find situations where CFI/F unreasonably estimates this active pool of soil organic matter.

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